

Amendments to the Specification:

Please insert the following Section Heading and paragraph on page 1, immediately below the title.

CROSS-REFERENCE TO RELATED APPLICATIONS

This is a submission to enter National Stage under 35 U.S.C. 371 for PCT Application No. PCT/IB2005/001771, filed on March 31, 2005 and published in English on October 13, 2005 as WO 2005/095625 A1, which claims priority to U.S. Provisional Patent Application No. 60/558,609, filed on March 31, 2004, all of which are incorporated by reference in their entirety to the extent not inconsistent with the disclosure herewith.

Please replace paragraph [0040] with the following amended paragraph:

[0040] This invention provides a method for making an AGP composition useful for fostering somatic embryogenic competence comprising: providing embryogenic callus; and harvesting AGP from said embryogenic callus. This invention provides a method for making an AGP composition useful for fostering somatic embryogenic competence comprising: expressing a protein or peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOS:1-7, 25, 26 ~~15, 17~~, and portions thereof; and harvesting the protein or peptide.

Please replace paragraph [0048] with the following amended paragraph:

[0048] In an embodiment of this invention, the pro-embryogenic AGP composition comprises a protein or peptide having a sequence of SEQ ID NO: 15 or SEQ ID NO:17, capable of being encoded by SEQ ID NOS: 14 or 16, a portion of at least fifteen amino acids of SEQ ID NO: 15 or 17, or having at least 80% sequence similarity to SEQ ID NOS: 15 or 17, or a protein having at least 80% sequence similarity to SEQ ID NOS: 25 or 26 or a tryptic thrombin digest thereof.

Please replace paragraph [0052] with the following amended paragraph:

[0052] FIG. 3 is a graph showing the percentage of embryogenic explants after four, six, and eight weeks of contact with non-embryogenic AGP, for four trials, as described in Example 6. The control, no contact with non-embryogenic AGP, is solid striped, and contact with non-embryogenic AGP is striped solid.

Please replace paragraph [0053] with the following amended paragraph:

[0053] FIG. 4 is a graph showing the percentage of embryogenic explants after four, six, and eight weeks of contact with AGP, for five four trials, as described in Example 7. The control, no contact with AGP, is striped, and contact with gum Arabic AGP is solid.

Please replace paragraph [0055] with the following amended paragraph:

[0055] FIG. 6 is a chart showing quantitation, by absorbance at 215 nm, of embryogenic callus AGPs eluting from a RP-HPLC column over time in minutes, as described in Example 9. The vertical line at 15 minutes, about 20% acetonitrile, denotes a separation between the hydrophilic (~~left-pointing arrow~~) and hydrophobic (~~right-pointing arrow~~) fractions.

Please replace paragraph [0057] with the following amended paragraph:

[0057] FIG. 8 is a chart showing quantitation, by absorbance at 215 nm, of embryogenic callus AGPs eluting off of a RP-HPLC column over time in minutes, as described in Example 11. Four peaks are labeled. ~~Time points used to begin and end collection of each peak are shown.~~

Please replace paragraph [0061] with the following amended paragraph:

[0061] FIG. 12 shows an illustration of the protein domain structure of the AGP backbone having sequences of SEQ ID NOS:18 or 20 ~~8 or 9~~, as described in Example 25. The AGP is divided into four domains: signal sequence (1), phytocyanin-like (2), pro-rich (3), and hydrophobic C-terminal (4).

Please delete paragraph [0063].

Please replace paragraph [0087] with the following amended paragraph:

[0087] The phrase, "fiber-producing plants" is used as in the art and is intended to include cotton, kenaf, ~~milkweed~~, flax, hemp, nettle, hop, and milkweed.

Please replace paragraph [0119] with the following amended paragraph:

[0119] This invention provides a method for making an AGP composition useful for fostering somatic embryogenic competence comprising: expressing a protein or peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOS:1-7, 25 and 26 ~~15 and 17~~; and harvesting the protein or peptide. This invention provides a method for making an AGP composition useful for fostering somatic embryogenic competence comprising: expressing a protein comprising a peptide having a sequence selected from the group consisting of SEQ ID NOS:1-7, ~~and SEQ ID NOS:15 and 17~~ and SEQ ID NOS: 25 and 26 and tryptic thrombin digests thereof, and harvesting the protein or peptide. In an embodiment of this invention, the protein or peptide is expressed in a plant host or a non-plant host, as is known in the art.

Please replace paragraph [0133] with the following amended paragraph:

[0133] In an embodiment of this invention, the AGP composition comprises a protein or peptide having a sequence of SEQ ID NOS: ~~15, 17, 25 or 26, capable of being encoded by SEQ ID NOS: 14 or 16, of a portion or at least about fifteen amino acids of SEQ ID NOS:15 or 17, or having 80% sequence similarity to SEQ ID NOS:15 or 17 or a tryptic thrombin digest of SEQ ID NOS: 25 or 26~~. In an embodiment of this invention, the AGP composition has a sequence of a phytocyanin-like domain (e.g., proteins PL-1 (SEQ ID NO:25) or PL-2 (SEQ ID NO:26) or a tryptic thrombin digest thereof) or a pro-rich domain (e.g., amino acids 139-156 of SEQ ID NO:15 18 and amino acids 131-182 of SEQ ID NO:17 20). In an embodiment of this invention, the protein or peptide is optionally engineered, not arabinosylated and/or glycosylated,

differently arabinosylated and/or glycosylated than the AGP from which it was derived, and/or chemically synthesized.

Please replace paragraph [0162] with the following amended paragraph:

[0162] Coker 315 hypocotyls were grown on hormone free media (basic media). After the callus was established, the explants, were transferred to basic media with or without added gum Arabic AGPs, gum Arabic is an exudate from *Acacia senegal* that is primarily AGP obtainable, e.g., from Sigma, St. Louis, MO, USA. ["] AGPs from gum Arabic were Yariv-precipitated and subsequently treated in the same way as the previously described AGP purification. The concentration of gum arabic AGP utilized was 1 mg/L.["] The explants were transferred to fresh media every four weeks. At time intervals, the callus was scored for embryogenic callus formation. ~~Data is shown in Table 5 of the percent of embryogenic explants. Between 93-108 explants were scored for each trial.~~ Table 4 and FIG. 4 show the results of four experiments. Between 71-135 explants were scored for each trial. Gum Arabic has no somatic embryogenic competence fostering activity and appeared to impede somatic embryogenic competence slightly.

Please replace paragraph [0165] with the following amended paragraph:

[0165] The total embryogenic AGPs extracted in Example 2 were split into the hydrophilic and hydrophobic fractions by RP-HPLC as in Example 3, but using a semi-preparative Zorbax 300 SB-C8 9.4 mm x 25 cm column and a flow rate of 3 mL/min. The embryogenic AGP peaks appeared in a bimodal distribution. The more hydrophilic fraction contained one major peak. The more hydrophobic fraction contained three peaks. The hydrophilic fraction accounted for about 75-85% of the AGP in all four peaks, and the hydrophobic fraction accounted for 15-25% (see FIG. 6). The two fractions were separated at about 15 minutes (see arrow vertical line on figure) or 20% acetonitrile. Other time points or acetonitrile concentrations that separate the peaks into the bimodal distribution are useful in the practice of this invention. In this example, the hydrophilic fraction was collected with the initial flow-through.

Please replace paragraph [0168] with the following amended paragraph:

[0168] The total embryogenic AGP extracted in Example 2 was split into 4 peaks (~~labeled by time point arrows~~) by RP-HPLC as in Example 9, as is shown in FIG. 8. Fraction 1, containing hydrophilic peak #1, the first peak to elute, was 75% of the total amount of AGP in the four peaks. Three hydrophobic peaks, Fraction 2 containing hydrophobic peak #1, Fraction 3 containing hydrophobic peak #2, and Fraction 4 containing hydrophobic peak #3, represented 4%, 11% and 10%, respectively, of the total AGP by weight. Fraction 1 containing hydrophilic peak #1 eluted at 4-12% acetonitrile or 3-9 min. Fraction 2 containing hydrophobic peak #1 eluted at 27-32% acetonitrile or 20-23.5 min. Fraction 3 containing hydrophobic peak #2 eluted at 32-37% acetonitrile or 23.5 to 28 min. Fraction 4 containing hydrophobic peak #3 eluted at 44-49% acetonitrile or 33-37 min.

On page 45, please amend Table 12 as follows:

Table 12
AGP Fostering Somatic Embryogenic Competence in Sicala 40

	Control Cocktail-D	AGP
4 weeks	0%	11%
6 weeks	0%	16%
8 weeks	0%	16%

Please replace paragraph [0189] with the following amended paragraph:

[0189] Coker 315 petioles were grown on basic media without added hormones and with hormone cocktail A, B, C, D, or E (~~FIGS. 13A-13J~~). After five weeks, explants were transferred to fresh media with or without added AGPs extracted from embryogenic callus, Example 2. The concentration of AGP was about 2 mg/L. The explants were transferred to fresh media, with or without AGP, every four weeks. At time intervals, the explants were scored for callus formation and for embryogenic callus

formation. Callus survived for about four weeks longer in the presence of AGPs, but everything eventually died, except with hormone cocktail D, which became embryogenic regardless of the presence or absence of AGPs see FIGS. 13G and 13H).

Please replace paragraph [0190] with the following amended paragraph:

[0190] Coker 315 leaves were grown on basic media without added hormones and with hormone cocktail A, B, C, D, or E, and with or without AGP extracted from embryogenic callus, Example 2. After five weeks, explants were transferred to hormone free media with or without added AGPs extracted from embryogenic callus, Example 2. The concentration of AGP was about 2 mg/L. The explants were transferred to fresh media, every four weeks. At time intervals, the explants were scored for callus formation and for embryogenic callus formation. Callus was produced on several combinations of hormones as well as on hormone free media. Inclusion of the AGP in the media resulted in about 25% more rapid formation of embryogenic callus (FIGS. 14A–14C), or formation of an equivalent percentage of embryogenic callus in about six weeks instead of about eight weeks, depending on the hormone cocktail. FIG. 14A shows embryogenic callus produced after contacting with AGP containing media after transfer from callus induction media with AGP. FIG. 14B shows callus produced using hormone cocktail D followed by contacting with AGP. FIG. 14C shows embryogenic callus produced without AGP after callus induction using hormone cocktail B. Contacting leaves with AGP fostered somatic embryogenesis.

Please replace paragraph [0207] with the following amended paragraph:

[0207] The sequence of the protein comprising peptides having sequences in SEQ ID NOS:1-2 and 27 is listed in SEQ ID NO:18, and the gene has been named GhCAGP1, for *Gossypium hirsutum* chimeric AGP #1. The sequence of the protein comprising a peptide having the sequence in SEQ ID NO:5 is listed in SEQ ID NO:20, and the gene has been named GhCAGP2, for *Gossypium hirsutum* chimeric AGP #2. Both have four domains, as shown in FIG. 12 and as listed below: a signal sequence, a phytocyanin-like domain, a pro-rich domain, and a hydrophobic C-terminal tail. The

gene sequences encoding SEQ ID NOS:18 and 20 are listed in SEQ ID NOS:17 and 19, respectively. SEQ ID NO:1 corresponds to amino acid numbers 79-94 of SEQ ID NO:18; SEQ ID NO:2 corresponds to amino acid numbers 56-63 of SEQ ID NO:18; and SEQ ID NO:5 corresponds to amino acid numbers 33-38 of SEQ ID NO:20.

Please replace paragraph [0208] with the following amended paragraph:

[0208] The signal sequence is located at amino acids 1-25 (nucleotide bases 1-75). The phytocyanin-like domain is located at amino acids 26-138 (nucleotide bases 76-414). The pro-rich domain is located at amino acids 139-156 (nucleotide bases 415-468). The hydrophobic C-terminal tail is located at amino acids 157-175 (nucleotide bases 469-525). The peptides corresponding to and having sequences similar to SEQ ID NOS:1, 2 and 27 5 are shown in bold.

Please replace paragraph [0217] with the following amended paragraph:

[0217] AGPs were extracted from embryogenic Siokra 1-4 callus (method of Example 2). The HPLC profile was compared to pro-embryogenic Coker AGPs (FIG 13-17). The profiles were similar, except that Hydrophobic Peak #3 had a slightly different retention time and shape, but this peak is slightly variable in extractions from Coker.